Clin Exp Immunol 1995; **102**:186–191

Beneficial effect of amrinone on murine cardiac allograft survival

T. HIROZANE, A. MATSUMORI, Y. FURUKAWA, S. MATSUI, Y. SATO,
Y. MATOBA & S. SASAYAMA *Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan*

(Accepted for publication 14 June 1995)

SUMMARY

Amrinone is a non-glycoside positive inotropic agent with an inhibitory effect on a cyclic adenosine monophosphate (AMP) phosphodiesterase isoenzyme. In the present study, we examined the immunosuppressive action of amrinone, since several other cyclic AMP-elevating agents have been shown to suppress T lymphocyte activation. First, the *in vivo* effects of amrinone were investigated. Oral amrinone treatment, at 40 mg/kg per day, significantly prolonged median cardiac allograft survival compared with non-treated controls (22·0 days *versus* 10·5 days, $P < 0{\cdot}01$) when DBA/2 mouse hearts (H-2^{d}) were heterotopically transplanted into C57Bl/6 mice (H-2^{b}). Histopathological examination showed that there was less prominent cellular infiltration in the amrinone-treated than in the non-treated allografts. Plasma amrinone concentrations of mice after a single oral dose of 40 mg/kg were within the range of clinical relevance. To clarify the mechanism of action, *in vitro* studies were done. The generation of specific cytotoxic T lymphocytes after mixed lymphocyte culture was significantly suppressed by addition of amrinone to the culture medium at 5 μg/ml. The production of IL-2 and the interferon-gamma during mixed lymphocyte culture was also suppressed by amrinone at 5 μg/ml. However, the level of intracellular cyclic AMP in mouse splenic lymphocytes was not affected significantly by the same dose of amrinone. In conclusion, amrinone has immunosuppressive actions at the therapeutic doses, and it may be a beneficial agent for therapy against acute cardiac allograft rejection.

Keywords amrinone immunosuppression cardiac transplantation cytotoxic T lymphocytes cytokine

INTRODUCTION

Amrinone, 5-amino-3,4′-bipyridine-6(1H)-one, is a non-glycoside cardiotonic agent with positive inotropic properties in the mammalian cardiac muscles [1–3]. Previous studies have suggested that the drug exerts its positive inotropic effect through inhibition of the cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE)-III [4,5].

Amrinone was developed to provide orally administered positive inotropic therapy in the management of chronic heart failure. However, long-term oral administration of the drug, as well as having limited clinical effectiveness, has been shown to elicit a considerable number of intolerable side effects including gastrointestinal complaints, liver dysfunction, and thrombocytopenia [6]. On the other hand, the short-term i.v. use of the drug provides acute beneficial haemodynamic changes in patients with heart failure [7–9]. Because of the clinical results, further development of the orally administered form of amrinone was stopped, whereas the i.v. form was approved for clinical use [10].

From the 1980s, it has been suggested that amrinone has possible target cells other than cardiac myocytes. For instance, the inhibiting effect of amrinone on platelets is well known. Thrombocytopenia has been shown to occur in 15–20% of patients treated orally, although it has been reported in only 2·4% of patients with short-term parenteral therapy [8]. In addition, macrophages and neutrophilic leucocytes have been suspected to be other targets [11,12]; however, possible action of amrinone on other cells remains to be investigated.

Since many researchers have demonstrated that cAMP-elevating agents inhibit the proliferation and function of human T lymphocytes [13–15], we investigated in the present study whether amrinone has an immunosuppressive action, and so may be useful for therapy against acute cardiac allograft rejection. First, the effects of amrinone on heterotopically transplanted mouse cardiac allografts were investigated. Second, to confirm whether the dose of amrinone used in this

Correspondence: Akira Matsumori MD, Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, 54 Kawaracho, Shogoin, Sakyo-ku, Kyoto 606, Japan.

study was appropriate, plasma concentrations of the drug were measured in mice of the recipent strain. Third, the influence of amrinone on cytotoxic T lymphocyte activity (CTL) and on the production of IL-2 and interferon-gamma (IFN-γ) in mixed lymphocyte culture (MLC) was assessed. Finally, the effect of amrinone on the level of cAMP in mouse splenic lymphocytes was examined.

MATERIALS AND METHODS

Animals

Adult male DBA/2 (H-2^d), C57Bl/6 (H-2^b), and C3H/He (H-2^k) mice, 7–9 weeks old, were obtained from the Shizuoka Agricultural Cooperative Association (Shizuoka, Japan). Mice were housed in stainless steel cages with controlled light/dark cycles and provided with food and water *ad libitum*.

Transplantation

DBA/2 mice served as donors and C57Bl/6 mice as recipients. Heterotopic cardiac transplantation was done using a modification of the method described by Corry *et al.* [16]. Ischaemic time was routinely 45–60 min, with a success rate of approximately 80%. Technical failures within the first 72 h were excluded from the experiment. Viability of the cardiac allografts was assessed by daily abdominal palpation and confirmed by electrocardiograms. The day of rejection was defined as the day of cessation of heartbeat.

Amrinone

Amrinone was generously provided as a pure powder by Yamanouchi Pharaceutical Co. (Tokyo, Japan). Amrinone was dissolved in 0·5 N lactic acid, and then diluted with distilled water to a final concentration of 5 mg/ml. The drug was administered by transoesophageal gavage as a single daily dose of 10 or 40 mg/kg, beginning on the day of transplant and continuing for 60 days when allografts kept beating.

Histopathological examination

The cardiac allografts were harvested on post-transplant day 5 or 10, since our preliminary experiments showed that apparent cellular infiltration emerged in DBA/2 allografts on post-transplant day 4 or 5, whereas maximal infiltration was seen on days 9–11, the period during which the allografts were totally rejected. Harvested allografts were sectioned transversely at the maximal circumference of the ventricle and fixed in 10% formalin. The graft tissues were embedded in paraffin, and then stained with haematoxylin and eosin. Histological findings were scored blindly by two observers who were unaware of any background data. Cellular infiltration, necrosis, and interstitial haemorrhage and oedema were graded to determine the severity using a scale of 0 (none), 1 (trace), 2 (mild), 3 (moderate) and 4 (severe).

Measurement of plasma concentration of amrinone

To confirm whether the dose of amrinone used in this study was appropriate, plasma concentrations of the drug were measured in mice. Amrinone (40 mg/kg as single dose) was administered by transoesophageal gavage to male 8-week-old C57Bl/6 mice. The mice were killed and plasma samples were collected 2, 4, 8, and 24 h after administration. The concentration of amrinone in plasma was determined by using a high performance liquid chromatographic (HPLC) method as previously described [17] at the Bio Medical Laboratory Co. (Tokyo, Japan).

Assay of CTL activity

CTL culture and assay were done as previously described [18,19]. The spleens of the mice were removed and gently dissociated into single-cell suspensions in RPMI 1640 (Gibco, Grand Island, NY). Erythrocytes were lysed by ACK lysing buffer (0·15 M NH_4Cl, 1·0 mM $KHCO_3$, 0·1 mM Na_2EDTA pH 7·4) and removed from the lymphocyte populations by centrifugation (25°C, 20 min, 1500 ***g***) over a high-density solution of Lympholyte-M (Cedarlane, Hornby, Ontario, Canada). Cytoxic effector cells were generated during 5-day incubation in a bulk culture of 7×10^6 splenic C57Bl/6 responder cells (recipient strain) with 5×10^6 DBA/2 stimulators (donor strain) treated with mitomycin C (25 μg/ml; Sigma, St Louis, MO). Effector activity was determined by adding the cytotoxic cells to ^{51}Cr-labelled DBA/2 splenic target cells (donor strain) or C3H/He targets (third party), both of which were pre-stimulated with 10μg/ml lipopolysaccharide (LPS; Difco, Detroit, MI) for 2 days. Target cells (1×10^4) were incubated with the C57Bl/6 effector cells in round-bottomed 96-well tissue culture plates in triplicate. The employed effector:target (E:T) ratios were 100:1, 50:1 and 25:1, and the total volume was adjusted to 200 μl per well. After incubation at 37°C in 5% CO_2 in humidified air for 4 h, 100 μl of the supernatant were harvested from each well and radioactivity was measured with an automatic gamma scintillation counter. Spontaneous release was < 30% of the total incorporated counts. Specific cytotoxicity was calculated as:

$$\text{Specific cytotoxicity} = \frac{\text{(experimental ct/min} - \text{spontaneous ct/min}}{\text{(maximal ct/min} - \text{spontaneous ct/min)}} \times 100$$

Maximal ct/min was determined by 0·1% Triton X-100 (Nacalai Tesque, Kyoto, Japan) treatment of the ^{51}Cr-labelled target cells. All cells were cultured in RPMI 1640 with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin sulphate, 50 μM 2-mercaptoethanol (2-ME), and 2 mM glutamine (complete RPMI 10). For *in vitro* use, amrinone was dissolved in 0·5 N lactic acid and then diluted with distilled water to a final concentration of 1 mg/ml in 0·025 N lactic acid. The drug was added to the coculture medium at a concentration of 2·5 or 5 μg/ml. Controls were sham treated with the solvent.

Assay of cytokines

The MLC was performed as described above, using a responder:stimulator ratio of 7:5. The total number of lymphocytes was adjusted to 1×10^7/well. Amrinone was added to the medium at a concentration of 5μg/ml. Mouse IL-2 and IFN-γ concentrations in the culture supernatants were then determined by specific ELISA kits (Endogen Inc., Boston, MA). The sensitivity of the kit was > 3 pg/ml for IL-2 and > 15 pg/ml for IFN-γ. Supernatants were collected 12 h and 24 h after incubation.

Measurement of cAMP content

The level of cAMP in mouse splenocytes was measured by ELISA. The C57Bl/6 mouse splenocytes (1×10^7 cells/ml) were collected as described above. Cells were incubated for 60 min at 37°C in an Eppendorf tube either with 5 μg/ml amrinone or with the solvent only. At the end of the incubation period, the medium was removed and the cells were lysed by addition of

200 μl cold 6% trichloroacetic acid (TCA), and collected into another Eppendorf tube. The tube was centrifuged at 1500 *g* for 10 min. TCA in the supernatant was then removed by extracting three times with three volumes of water-saturated diethyl ether, and cAMP content was determined by a commercially available ELISA kit (Amersham, Aylesbury, UK).

Statistical analysis

Statistical analysis of the cardiac graft survival times and levels of cAMP employed the Mann–Whitney *U*-test. Scores for histological findings, cytolytic activities in the CTL assay, and levels of IL-2 and IFN-γ in culture supernatants were compared by one-factor ANOVA, since the data were parametrically distributed.

RESULTS

Effects of amrinone on cardiac allograft survival

Figure 1 shows the per cent survival of non- and amrinone-treated allografts. Non-treated C57Bl/6 mouse recipients ($n = 6$) rejected DBA/2 cardiac allografts with a median survival time of 10·5 days. Treatment with 10 or 40 mg/kg per day of amrinone (each $n = 6$) prolonged median graft survival to 12 or

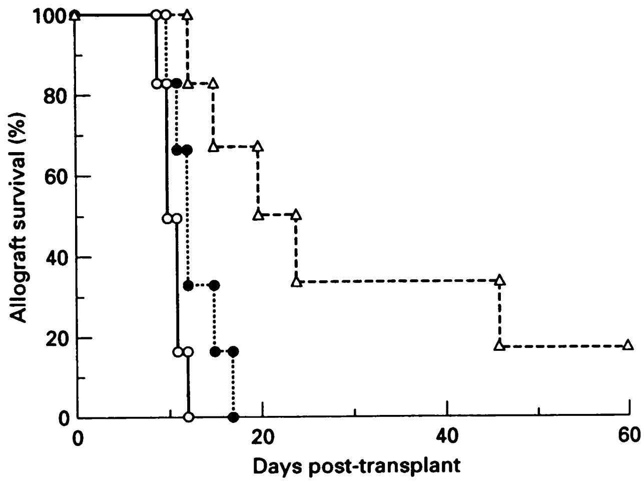

Fig. 1. Effect of amrinone on cardiac allograft survival. Oral amrinone treatment at a dose of 40 mg/kg per day (△) significantly prolonged cardiac allograft survival ($P < 0.01$ *versus* non-treated control (○)). However, treatment with the drug at 10 mg/kg per day (●) resulted in an insufficient effect ($P = \text{NS}$ *versus* non-treatment control).

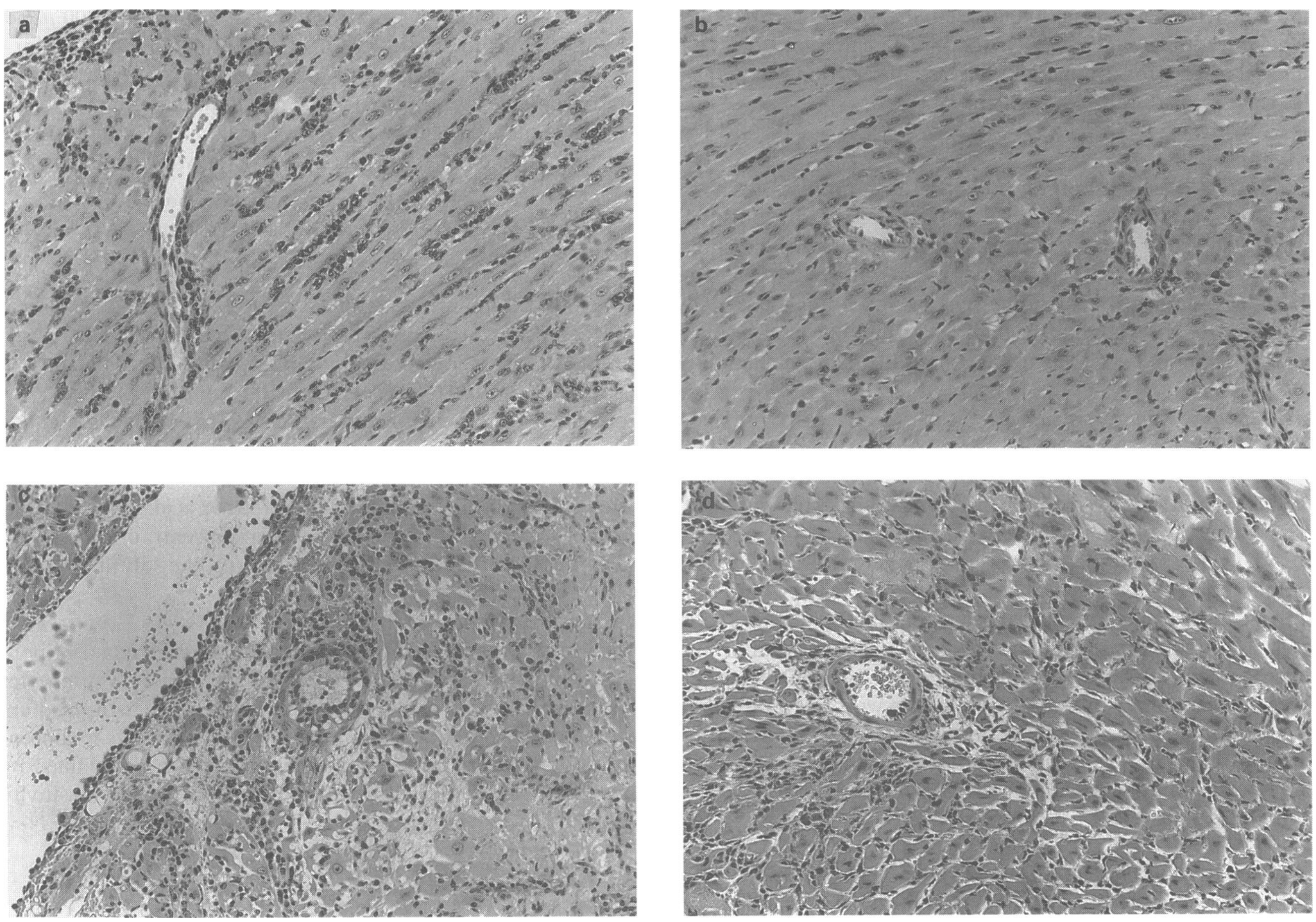

Fig. 2. Sections of mouse cardiac allografts. (a) Five-day-old non-treated allograft. (b) Five-day-old amrinone-treated allograft. (c) Ten-day-old non-treated allograft. (d) Ten-day-old amrinone-treated allograft. Mild interstitial mononuclear cell infiltration was observed in the 5-day-old allografts, whereas severe cell infiltration, interstitial haemorrhage and oedema, massive necrosis, and vascular inflammation were seen in the 10-day-old allografts. Interstitial mononuclear cell infiltration seemed to be less prominent in the amrinone-treated than in the non-treated allografts in each case. Amrinone was administered orally at a dose of 40 mg/kg per day. (H & E; magnification (a–d) × 200).

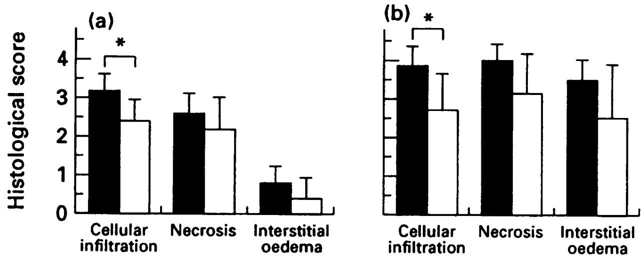

Fig. 3. Histological scores for cardiac allografts. Histological grading was done as described in Materials and Methods. The scores were based on allografts harvested on the post-transplantation day 5 (a) and day 10 (b). Each bar represents mean ± s.d. of five or six grafts. $P<0.05$ by ANOVA. ■, Control; □, amrinone.

22 days. Graft survival times was significantly prolonged in mice treated with 40 mg/kg per day amrinone ($P<0.01$). Although slightly prolonged graft survival was observed in mice treated with 10 mg/kg per day, the difference was not statistically significant.

Histological findings

Histological examination was done on DBA cardiac allografts transplanted into C57Bl/6 mice and harvested on post-transplantation day 5 or 10. Amrinone was administered orally at a dose of 40 mg/kg per day. As shown in Figs 2a,b, inflammatory changes observed in the 5-day-old grafts appeared to be less prominent in the amrinone-treated ($n=5$) than in the non-treated ($n=5$) allografts. The 10-day-old allografts generally displayed more extensive inflammation than the 5-day-old allografts; however, as shown in Figs 2c,d, cellular infiltration was milder in the amrinone-treated ($n=5$) than in non-treated allografts ($n=6$). The results of histological grading are summarized in Fig. 3a (5-day-old allografts) and in Fig. 3b (10-day-old allografts). The score of cellular infiltration was in each case significantly lower in the amrinone-treated than in the non-treated allografts.

Plasma concentration of amrinone

A single dose of amrinone, at 40 mg/kg, was administered by gavage to non-operated C57Bl/6 mice, and then concentrations of amrinone in plasma were measured. As shown in Table 1, peak plasma level was observed in samples harvested 2 h after administration, and values ranged from 2·19 to 3·90 μg/ml. The half life of the drug seemed to be less than 2 h. Taking into account that the therapeutic range of amrinone in human plasma is 0·6–6·4 μg/ml [9], the dose of 40 mg/kg may well be regarded as appropriate for mice.

Table 1. Plasma concentrations of amrinone in mice

Time (h)	n	Concentration (μg/ml, mean ± s.e.m.)
2	3	2·87 ± 0·53
4	3	0·40 ± 0·09
8	3	< 0·10*
24	3	< 0·10*

Amrinone was administered orally as a single dose of 40 mg/kg.
*Lower value than the minimal sensitivity (0·10 μg/ml).

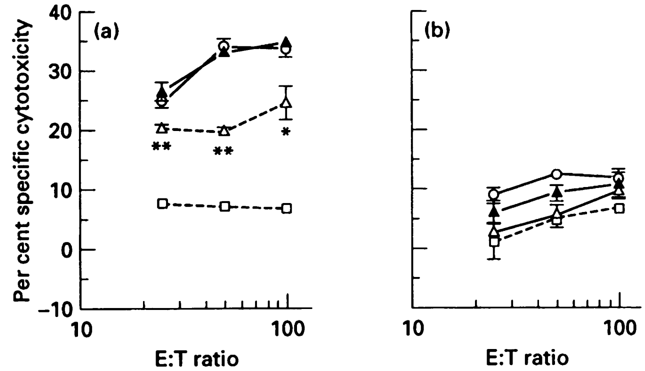

Fig. 4. Inibition of cytoxic T cell generation by amrinone. Splenocytes from C57Bl/6 mice were stimulated with DBA/2 cells for 5 days, and then tested against DBA/2 (a) or C3H/He cells (b). The bars represent cytolytic activity at E:T ratios of 25:1, 50:1 and 100:1. Amrinone, at a concentration of 2·5 (▲) or 5 μg/ml (△), was added to the medium during the 5-day coculture. Control C57Bl/6 splenocytes (○) were sham-treated with the solvent. Non-sensitized effectors (□) were C57Bl/6 splenocytes harvested on the day of cytolytic assay. Values are expressed as mean ± s.e.m. of triplicate cultures. Similar results were obtained in two other experiments. $^*P<0.05$; $^{**}P<0.01$ *versus* control by ANOVA.

Effects of amrinone on CTL activity in vitro

To clarify the mechanism of action of amrinone in allograft rejection, we examined its effects on CTL, measuring specific lysis of C57Bl/6 splenocytes (recipient strain) on DBA/2 splenocytes (donor strain) or on C3H/He splenocytes (third party). Compared with non-sensitized C57Bl/6 cells, the sham-treated control effector cells showed enhanced lytic activity to DBA/2 target cells after 5-day MLC, whereas effector cells treated with 5 μg/ml of amrinone displayed significantly lower CTL activity at E:T ratios of 25:1, 50:1 and 100:1 (Fig. 4a). However, treatment at 2·5 μg/ml failed to suppress lytic activity. These C57Bl/6 effector cells displayed little cytolytic activity when the target cells were from a third strain (Fig. 4b), suggesting that the lytic activity induced by MLC was specific to DBA/2 targets. These results indicate that amrinone may well inhibit

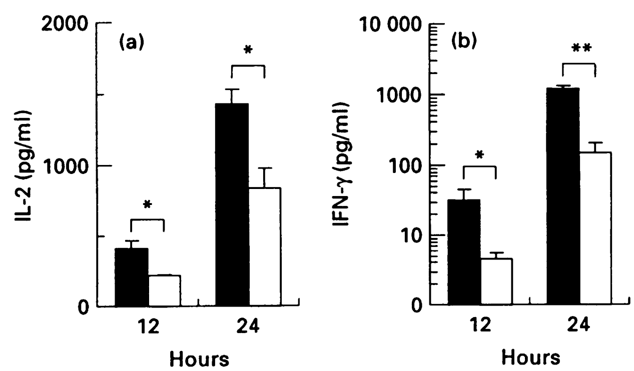

Fig. 5. Inibition of cytokine production by amrinone. (a) The concentration of IL-2 in the supernatants of mixed lymphocyte cultures was assayed using an ELISA kit, as described in Materials and Methods. The results represent two experiments. Values are expressed as mean ± s.e.m. of three mixed lymphocyte cultures. (b) The concentration of IFN-γ in supernatants was similarly assayed. $P<0.05$; $^{**}P<0.01$ by ANOVA. ■, Control; □, amrinone.

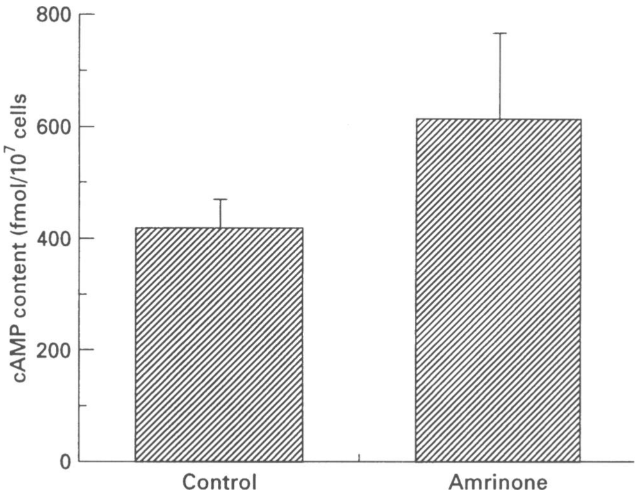

Fig. 6. Effect of amrinone on level of cAMP in mouse splenocytes. The level of cAMP in mouse splenocytes was measured by enzyme immunoasssay as described in Materials and Methods. The cells were treated with 5 μg/ml amrinone or with the solvent. Values are expressed as mean ± s.e.m. of three experiments.

the generation of CTL activity *in vivo* at therapeutic plasma levels.

Effects of amrinone on IL-2 and IFN-γ production

The concentrations of IL-2 and IFN-γ in the supernatants of allogenic one-way MLC were assayed 12 h and 24 h after incubation. Amrinone was added to the culture medium at a concentration of 5 μg/ml. As shown in Fig. 5a,b, the levels of both IL-2 and IFN-γ were significantly suppressed by amrinone at 12 h and 24 h after incubation, with the level of IFN-γ in the amrinone-treated group at 12 h being below kit sensitivity. The suppressive effect seemed to be more marked on IFN-γ than on IL-2 production.

Effect of amrinone on cAMP of lymphocytes

To confirm whether amrinone exerted an inhibitory effect on PDE at the concentration used (5 μg/ml), the levels of cAMP in mouse splenocytes were measured. As shown in Fig. 6, the mean level of cAMP in mouse lymphocytes treated with 5 μg/ml amrinone was approximately 1·5-fold higher than that in control, but the difference was not statistically significant.

DISCUSSION

The present study has demonstrated that amrinone exerted its *in vitro* immunological effects at 5 μg/ml, a concentration within the therapeutic plasma range. The dose required to display its *in vivo* effect, however, needs further elaboration. A dose of 40 mg/kg per day is about 2·4-fold higher than the dose used in humans for short-term therapy of congestive heart failure and about 4·9–8·3-fold higher than that used for therapy of chronic heart failure [6,7]. However, on the basis of body surface area, a given dose in mice is comparable to a dose that is about 12 times lower in humans [20]. Thus, a dose of 40 mg/kg per day in mice is equivalent to a dose of 3·3 mg/kg per day in humans, a dose within the therapeutic range. The plasma amrinone concentration measured in mice appears to support this equivalence.

The principal problem in the present study is that the drug was administered by the oral route. As described in the Introduction, long-term oral administration of amrinone has been shown to be complicated by a number of side effects as well as limited effectiveness. However, it is important to note that the mean daily dose of orally administered amrinone in the clinical study was 355 mg or 1·6 mg/kg tid (4·8 mg/kg per day) and the maximal daily dose was 600 mg [6]. These doses may be a little too high for immunosuppressive use, since the dose used in this study appears to be equivalent to a daily dose of 244 mg (3·3 mg/kg per day) in humans. In the present study, all of the recipient mice treated with amrinone showed good weight gain after transplantation, and no death was observed after post-transplantation day 2, indicating that, in mice, fatal complications did not occur at a dose of 10 or 40 mg/kg per day of amrinone.

As stated in the Introduction, the action of amrinone on cardiac myocytes has been suspected to be based on the inhibition of PDE-III. The inhibitory effect of amrinone on lymphocyte activation may also be due to its inhibition of PDE, since several investigators have established that analogues and agents that raise intracellular cAMP inhibit the proliferation and function of T cells [13–15]. However, whether amrinone increases the level of intracellular cAMP at the therapeutic plasma levels has not been clearly established. For instance, it has been shown that amrinone, in amounts which produce a significant increase in the force of the isolated cat atrial contractions (100 μg/ml), does not increase the level of cAMP in cardiac tissue [2]. In the present study, the level of cAMP in mouse lymphocytes was not affected significantly by treatment with amrinone at 5 μg/ml, the concentration at which lymphocyte functions were suppressed. Taken together, it is doubtful that the PDE-inhibiting action of amrinone is closely associated with its immunosuppressive property at therapeutic plasma levels.

Other actions of the drug may play a role in promoting cardiac allograft survival. For instance, amrinone has been shown to dilate vessels and thereby decrease vascular resistance [21]. Through this vasodilator action, the drug may improve blood supply to ischaemic parts of allografts undergoing rejection. In addition, previous research has shown that amrinone inhibits the release of thrombin-induced, platelet-derived growth factor-like protein from human endothelial cells [22]. The endothelial stabilizing effect may also play a role in promoting allograft survival, since severe vasculitis was observed in the 10-day-old cardiac allografts (Fig.2c). These effects of amrinone on vascular function may be important in comparing the drug with cyclosporin A (CsA), the first-choice drug available for prevention of acute graft rejection. The immunosuppression induced by high-dose CsA therapy may be greater than that by therapeutic doses of amrinone. However, CsA has been shown to have a considerable number of toxic side-effects with the main effects on both renal and vascular function [23–24]. For instance, CsA has been shown to provoke contraction and hyperplasia of small arteries, together with decreased renal cortical flow and glomerular filtration rate [25]. Therefore, amrinone may be indicated for patients who are sensitive to the CsA toxicity, since it may act beneficially by dilating and stabilizing the vasculature, as stated above.

It is of note that amrinone has a direct promoting action on the sodium–calcium exchange channel in the plasma membrane of cardiac myocytes, resulting in increased entry of calcium ions [8]. This channel-directed action of amrinone is independent of its PDE-inhibiting action; however, the role of this action in its immunosuppressive effect is uncertain.

The results of the present study have demonstrated that amrinone, at a therapeutic concentration, inhibits IL-2 and IFN-γ production during allogeneic MLC. This finding may be in accordance with those in a previous report, which demonstrated that amrinone suppressed tumour necrosis factor (TNF) production by lipopolysaccharide-stimulated mouse macrophages [11]. The expression of some cytokines, including IFN-γ, IL-2, and TNF-α, has been shown to be elevated in organ allografts undergoing acute rejection [26,27]. These cytokines could play a role in the pathogenesis of acute rejection through the interplay of effector cells or through direct injury of donor organs [28]. Amrinone may promote cardiac allograft survival by suppressing these inflammatory cytokines.

In conclusion, amrinone is a unique drug that has positive inotropic, vasodilatory, and immunosuppressive actions. Of course, amrinone may not be effective and, when given orally, may provoke a number of side effects as stated in the Introduction; however, its possible clinical usefulness for prevention of acute cardiac allograft rejection is worthy of notice and needs further investigation.

ACKNOWLEDGMENTS

This work was supported by a research grant from the Ministry of Health and Welfare of Japan, and a grant-in-aid for general scientific research from the Ministry of Education, Science, and Culture of Japan. We would like to thank Yamanouchi Pharmaceutical Co. (Tokyo, Japan) for generous gifts of amrinone.

REFERENCES

1 Farah AE, Alousi AA. New cardiotonic agents: a search for a digitalis substitute. Life Sci 1978; **22**:1139–48.

2 Alousi AA, Farah AE, Lesher GY, Opalka CJ. Cardiotonoc activity of amrinone – Win 40680 [5-amino-3,4′-bipyridin-6(1H)-one]. Circ Res 1979; **45**:666–77.

3 Benotti JR, Grossman W, Braunwald E, Davolos DD, Alousi AA. Hemodynamic assessment of amrinone: a new inotropic agent. N Engl J Med 1978; **299**:1373–7.

4 Endoh M, Yamashita S, Taira N. Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J Pharmacol Exp Ther 1982; **221**:775–83.

5 Leyen H. Phosphodiesterase inhibition by new cardiotonic agents: mechanism of action and possible clinical relevance in the therapy of congestive heart failure. Klin Wochenschr 1989; **67**:605–15.

6 Massie B, Bourassa M, DiBianco R, Hess M, Konstam M, Likoff M, Packer M. Long-term oral administration for congestive heart failure: lack of efficacy in a multicenter controlled trial. Circulation 1985; **71**:963–71.

7 Klein NA, Siskind SJ, Firshman WH, Sonnenblick EH, LeJemtel TH. Hemodynamic comparison of intravenous amrinone and dobutamine in patients with chronic congestive heart failure. Am J Cardiol 1981; **48**:170–5.

8 Mancini D, LeJemtel T, Sonnenblick E. Intravenous use of amrinone for the treatment of the failing heart. Am J Cardiol 1985; **56**:8b–15b.

9 Tanaka K, Takano T, Seino Y. Effects of intravenous amrinone on heart failure complicated by acute myocardial infarction: comparative study with dopamine and dobutamine. Jpn Circ J 1986; **50**:652–8.

10 Leier CV. Current status of non-digitalis positive inotropic drugs. Am J Cardiol 1992; **69**:120G–9G.

11 Giroir BP, Beutler B. Effect of amrinone on tumour necrosis factor production in edotoxic shock. Circ Shock 1992; **36**:200–7.

12 Blank I, Baudisch W, Burmeister J, Franz U. Die Wirkung von Amrinon und Trapidil auf Adharenz, Migration, und Phagozytoseverhalten neutophiler Granulozyten des Menschen. Acta Hamatol (Leipz) 1987; **114**:760–7.

13 Lingk DS, Chan MA, Gelfand EW. Increased cyclic adenosine monophosphate levels block progression but not initiation of human T cell proliferation. J Immunol 1990; **145**:449–55.

14 Coffino P, Gray JW, Tomkins GM. Cyclic AMP, a nonessential regulator of the cell cycle. Proc Natl Acad Sci USA 1975; **72**:878–82.

15 Valitutti S, Dessing M, Lanzavecchia A. Role of cAMP in regulating cytotoxic T lymphocyte adhesion and motility. Eur J Immunol 1993; **23**:790–5.

16 Corry RJ, Winn HJ, Russel PS. Primary vascularized allografts of hearts in mice. Transplantation 1973; **16**:344–50.

17 Kullberg MP, Dorrbecker B, Lennon J, Rowe E, Edelson J. High-performance liquid chromatographic analysis of amrinone and its N-acetyl derivative in plasma: pharmacokinetics of amrinone in the dog. J Chromatography 1980; **187**:264–70.

18 Kruisbeek AM. Isolation and fractionation of mononuclear cell populations. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, eds. Current protocols in immunology. New York; Green Publishing Associates and Wiley-Interscience; 1991: 3.1.1–5.

19 Wunderlich J, Shearer G. Induction and measurement of cytotoxic T lymphocyte activity. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, eds. Current protocols in immunology. New York: Green Publishing Associates and Wiley-Interscience; 1991: 3.11.1–7.

20 Chodera A, Feller K. Some aspects of pharmacokinetic and biotransformation differences in humans and mammal animals. Int J Clin Pharacol 1978; **16**:357–60.

21 Levy JH, Bailey JM. Amrinone: its effect on vascular resistance and capacitance in human subjects. Chest 1994; **105**:62–64.

22 Shaddy RE, Paisley JE, Hansen JC. Effects of amrinone on thrombin-induced platelet-derived growth factor-like protein secretion from endothelial cells. Pediatr Res 1991; **30**:351–54.

23 Mason J. Pathophysiology and toxicology of cyclosporine in humans and animals. Pharmacol Rev 1989; **42**:423–34.

24 Rego A, Vargas R, Seedabarum C, Kawahara M, Foeg ML, Ramwell PR. Systemic vascular effects of CyA treatment in normotensive rats. J Pharmacol Exp Ther 1991; **259**:905–15.

25 Cairns HS, Fairbanks LD, Westwick J, Neild GH. Cyclosporin therapy *in vivo* attenuates the response to vasodilators in the isolated perfused kidney of the rabbit. Br J Pharmacol 1989; **98**:463–8.

26 Arbustini E, Grasso M, Diegoli M *et al.* Expression of tumor necrosis factor in human acute cardiac rejection. Am J Pathol 1991; **139**:709–15.

27 Dallman MJ, Larsen CP, Morris PJ. Cytokine gene transcription in vascularized organ allografts. J Exp Med 1991; **174**:493–6.

28 Soulillou JP. Cytokines and transplantation. Transplant Proc 1993; **25**:106–8.